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Utilization of dual stable isotope markers ($\delta^{13}C$ and $\delta^{15}N$) to determine trophic structure in aquatic environment of Malaysian peat swamp forest

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Abstract

This study was conducted to develop a preliminary food web model of aquatic environment at the North Selangor Peat Swamp Forest (NSPSF) using stable isotope markers ($\delta^{13}C$ and $\delta^{15}N$). The samples were collected from two selected points of peat swamp area representing disturbed and undisturbed areas. Samples collected then underwent analytical procedures before being analysed using an Isotope Mass Ratio Spectrometry (IRMS). Results showed that there are differences in trends of the food web between disturbed and undisturbed area. A consumer in the higher trophic level had significantly higher $\delta^{13}C$ and $\delta^{15}N$ values compared to the lower consumer. *Hemibagus nemurus* has become the top predator in disturbed areas while *Channa lucius* becomes the top predator in undisturbed areas. Sampling throughout the year is highly recommended as different species are only available for a certain period of time due to the water input from the rainfall.

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Keywords: Stable isotope; peat swamp; aquatic ecosystem; food web; trophic level

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1. Introduction

Some scientists are concerned with the circulation of organic matters, especially when it comes to food web structures in aquatic ecosystems. Therefore, food web study is important as it contains vital details and information about what is actually happening in our surroundings. Furthermore, the relative importance of the potentially numerous organic matter sources and the nature of control linkages within food web in aquatic ecosystems are poorly understood, thus prevents deep understanding about functioning ecosystem and hinders all kinds of attempts to manage components communities [1]. Stable isotope is one of the best methods to characterize trophic structures. It has been used to predict and track the flow and concentration anthropogenic contaminants [2]. Peat swamp ecosystem is one of the important areas and the most interesting habitat that has high species endemism which needs to be studied [3]. Because of that, it has been selected as our study site. The objective of this study is to determine the suitability of stable isotope marker in developing a food web model of aquatic environments in North Selangor Peat Swamp Forest (NSPSF).

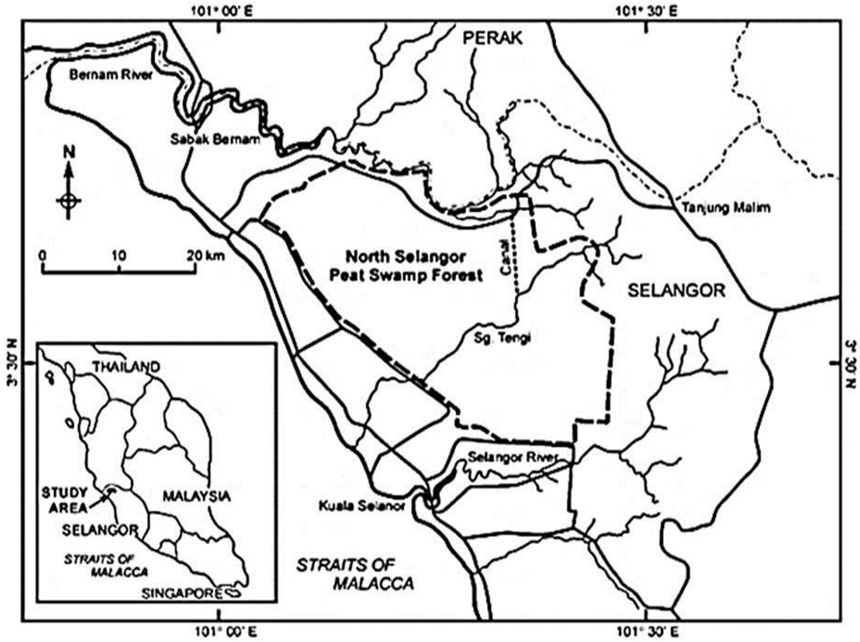

Fig. 1. Location of the North Selangor Peat Swamp Forest in state of Selangor, Malaysia [4]

2. Materials and methods

2.1. Sampling & storage

The sampling activity had been conducted twice, which is during December 2013 and January 2014. There are two selected points of peat swamp area that are located along Sungai Tenggi, Kuala Selangor. Each of the point represents two different circumstances, which are the disturbed and undisturbed area. These sampling areas are located at the Global Positioning System (GPS) coordinate of $3^0$28.473' N 101^0 13.308'E (disturbed area) and $3^0$30.238' N 101^0 16.237'E (undisturbed area). The fish samples were collected by fishing using fish rod, fish net and also by using wicker trap baited with oil palm nuts. All of the samples, including plants and soil samples were stored in an ice box in order to keep the samples in a good condition before being analyzed.

2.2. Analytical procedures

The samples obtained were rinsed with milli-Q water to remove any unnecessary foreign materials and particles and also for hygiene purposes in order to obtain more accurate results. The fishes were cut ventrally along the upper part of the pectoral fin, and the fillet tissue was removed into a clean centrifuged tube while the plant samples were chopped before being put into the centrifuged tube. All of the samples were then freeze dried in the freeze dryer for about 15 hours before being added to the mixture of chloroform and methanol with the ratio of 2:1 for about 3 hours to remove unnecessary lipid components. The samples were then centrifuged for about 10 minutes. Then, the supernatant then was discarded and the remaining pellet was put inside the soda lime glass. The pellets were dried for 1 hour for optimal drying. Then, the samples were fumed with 12 M for about 10 hours in order to remove inorganic carbonates that remain in the samples before being fumed with NaOH pellet for about 3 hours. Finally, the samples were oven dried at 60^{o}C for another 3 hours before being analyzed using Continuous-Flow Isotope Ratio Mass Spectrometer (CF-IRMS).

Nitrogen (N) and carbon (C) stable isotope compositions were measured using SERCON GEO 20-20 Continuous-Flow Isotope Ratio Mass Spectrometer (CF-IRMS). Generally, isotopic compositions of N and C were expressed in δ notation ($\delta^{15}N$, $\delta^{13}C$) as part per thousand (‰), where it is pronounced as “permill” as it is derived from Latin. The measurement precision was less than 0.2‰ for both $\delta^{15}N$ and $\delta^{13}C$. Stable isotope data are reported according to the equation (1):

$$\delta(‰) = \left[\frac{R_{sample}}{R_{s\tan dard}} - 1 \right] \times 1000 \tag{1}$$

where R is ^{15}N / ^{14}N or ^{13}C / ^{12}C of sample or standard, X is the $\delta^{15}N$ or $\delta^{13}C$ in (‰) from the deviation of the sample with the recognized isotope standards.

3. Results

There were 16 biological samples that had been collected in this study. Based on the results, it cleary shows that each of the sample potrays various $\delta^{15}N$ and $\delta^{13}C$ signatures. Table 1 and Table 2 summarize the N and C isotopic values in all of the samples that had been collected in North Selangor Peat Swamp Forest in the undisturbed and disturbed area respectively.

Table 1 Sample detail of organism from undisturbed area

Sample	$\delta^{13}C$	$\delta^{15}N$	TLE
	Mean ± SD	Mean ± SD	Mean ± SD
Hemibagrus nemurus	-26.30 ± 1.32	12.59 ± 0.63	3.84 ± 0.19
Channa lucius	-28.76 ± 1.44	14.18 ± 0.71	4.31 ± 0.21
Desmopuntius johorensis	-29.19 ± 1.46	11.58 ± 0.58	3.55 ± 0.17
Pristolepis grooti	-29.36 ± 1.47	13.73 ± 0.69	4.18 ± 0.20
Helastoma teminckii	-31.65 ± 1.59	8.64 ± 0.43	2.68 ± 0.13
Anabas testudinus	-26.08 ± 1.30	9.67 ± 0.48	2.99 ± 0.14
Rasbora dusonensis	-24.56 ± 1.22	8.88 ± 0.44	2.75 ± 0.13
Rasbora cephalotania	-27.13 ± 1.36	7.31 ± 0.37	2.29 ± 0.11
Clarias neuhofi	-28.52 ± 1.43	8.98 ± 0.45	2.78 ± 0.13
Myrtaceae sp.(cf)	-30.98 ± 1.55	3.76 ± 0.19	1.25 ± 0.06
Poaceae sp.	-28.55 ± 1.43	3.32 ± 0.17	1.18 ± 0.05
Soil	-29.82 ± 1.49	2.79 ± 0.14	0.96 ± 0.04

*TLE: Trophic Level Estimation

Table 2 Sample detail of organism from disturbed area

Sample	δ^{13}C	δ^{15}N	TLE
	Mean ± SD	Mean ± SD	Mean ± SD
Anabas testudinus	-26.88 ± 1.35	7.96 ± 0.40	2.48 ± 0.18
Channa lucius (S)	-26.63 ± 1.33	11.31 ± 0.57	3.47 ± 0.17
Channa lucius (B)	-28.60 ± 1.43	12.71 ± 0.64	3.88 ± 0.19
Hemibagrus nemurus	-26.32 ± 1.32	13.99 ± 0.70	4.26 ± 0.21
Pristolepis grooti	-25.82 ± 1.29	12.40 ± 0.62	3.79 ± 0.18
Sphaericthys osphromenoides	-29.81 ± 1.49	10.96 ± 0.55	3.36 ± 0.16
Parachela oxygastroides	-32.97 ± 1.65	11.74 ± 0.59	3.59 ± 0.17
Myrtaceae sp.(cf)	-28.95 ± 1.45	2.59 ± 0.13	0.90 ± 0.04
Melastoma malabatricum	-29.49 ± 1.47	3.10 ± 0.16	1.05 ± 0.05
Stenochlaena polustris	-25.00 ± 1.25	4.50 ± 0.23	1.46 ± 0.07
Soil	-30.78 ± 1.54	3.63 ± 0.18	1.21 ± 0.05

*TLE: Trophic Level Estimation

4. Discussion

Carbon isotopic compostion of animal reflects their diet within the range of about 1‰ [5], while the systematic enrichment of consumer's isotopic values of δ^{15}N is about 3-4 ‰ according to their diet [6]. Due to this basis, a trophic structure can be constructed within a graph as Y axis: δ^{15}N level, while X axis: δ^{13}C level. A baseline value is highly crucial in order to determine a trophic structure. Generally, δ^{15}N values of primary producer are the most suitable to be utilized as a baseline value. However, since there is no suitable sample from the peat swamp area that can be used as a baseline, the δ^{15}N values of clam from a wetland in China was used as a reference (δ^{15}N 6.40 ± 0.45) [7].

4.1. Undisturbed area

According to the isotopic signatures (Fig. 2), the highest trophic level is occupied by *Channa lucius* and *Pristolepis grooti (*TL 4), followed by *Desmopuntius johorensis* and *Hemibagrus nemurus* in trophic level 3 (TL 3), *Rasbora dusonensis*, *Rasbora cephalotania, Anabas testudinus*, *Clarias neuhofi* and *Helastoma teminkchii* in trophic level 2 (TL 2), species of Myrtaceae family and Poaceae family are in trophic level 1 (TL 1), while soil were found to be the lowest in trophic level among other samples due to its low δ^{15}N value. *Channa lucius* and *Pristolepis grooti* were most probably having other fishes as their main food preferences, due to presence of *Channa lucius* as it is reported to be piscivorous [8], proving that they were the highest in the trophic level due to the accumulation of nitrogen isotopic value from the organisms in the lower trophic level [9,10,11]. However, within the environment circumstances, we suggested that it is not possible that these two species were also having other food preferences such as detritus due to the low visibility within the blackwater. The mean values for δ^{15}N and δ^{13}C values for *Desmopuntius johorensis* are -29.19 ± 1.46‰ and 11.58 ± 0.58‰ respectively, while the mean δ^{15}N and δ^{13}C values for *Channa lucius* are -28.76 ± 1.44‰ and 14.18 ± 0.71‰ respectively. Since both of the organisms' δ^{15}N and δ^{13}C values are within the range of 3-4 ‰ and 0-1‰ respectively, it is likely that these two organisms are having prey-predator relationship.

4.2. Differences in food web pattern between disturbed and undisturbed area.

We can see the trend of food web differences between the disturbed and undisturbed peat swamp area. In the undisturbed area, the top predator is *Channa lucius* while in the disturbed area, the top predator is *Hemibagrus nemurus*. This is because different places have different fish assemblages due to biotic and abiotic factor of the surrounding environment. The same reason could be applied at the second and third trophic level of the disturbed and undisturbed area as the fish density is different in both of the trophic levels.

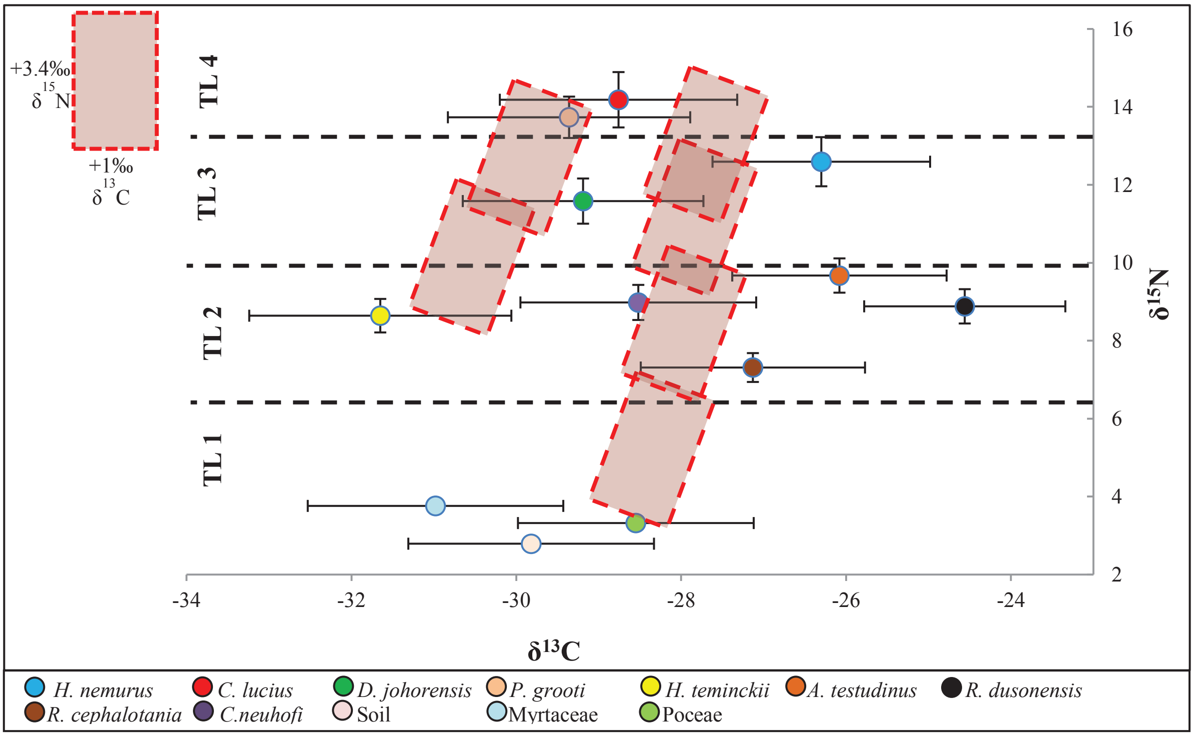

Fig. 2. Isotopic signatures of samples in undisturbed area. Dotted boxes indicates prey-predator relationship while TL refer to the trophic level of the species

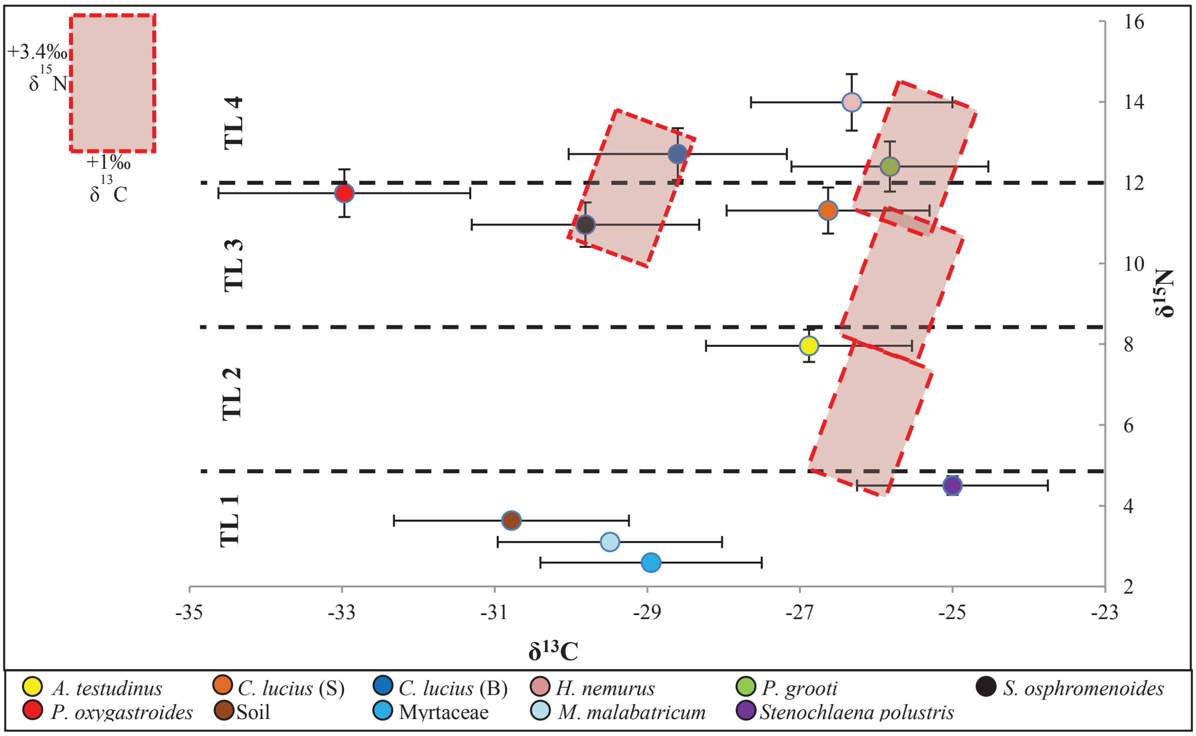

Fig. 3. Isotopic signatures of samples in undisturbed area. Dotted boxes indicates prey-predator relationship while TL refer to the trophic level of the species

5. Conclusion

Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) can demonstrate prey-predator relationship and even create a short food chain. The different trends in food web model in both disturbed and undisturbed area can clearly be seen as different places have different fish assemblages because of biotic and abiotic factor of the surrounding environment.

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